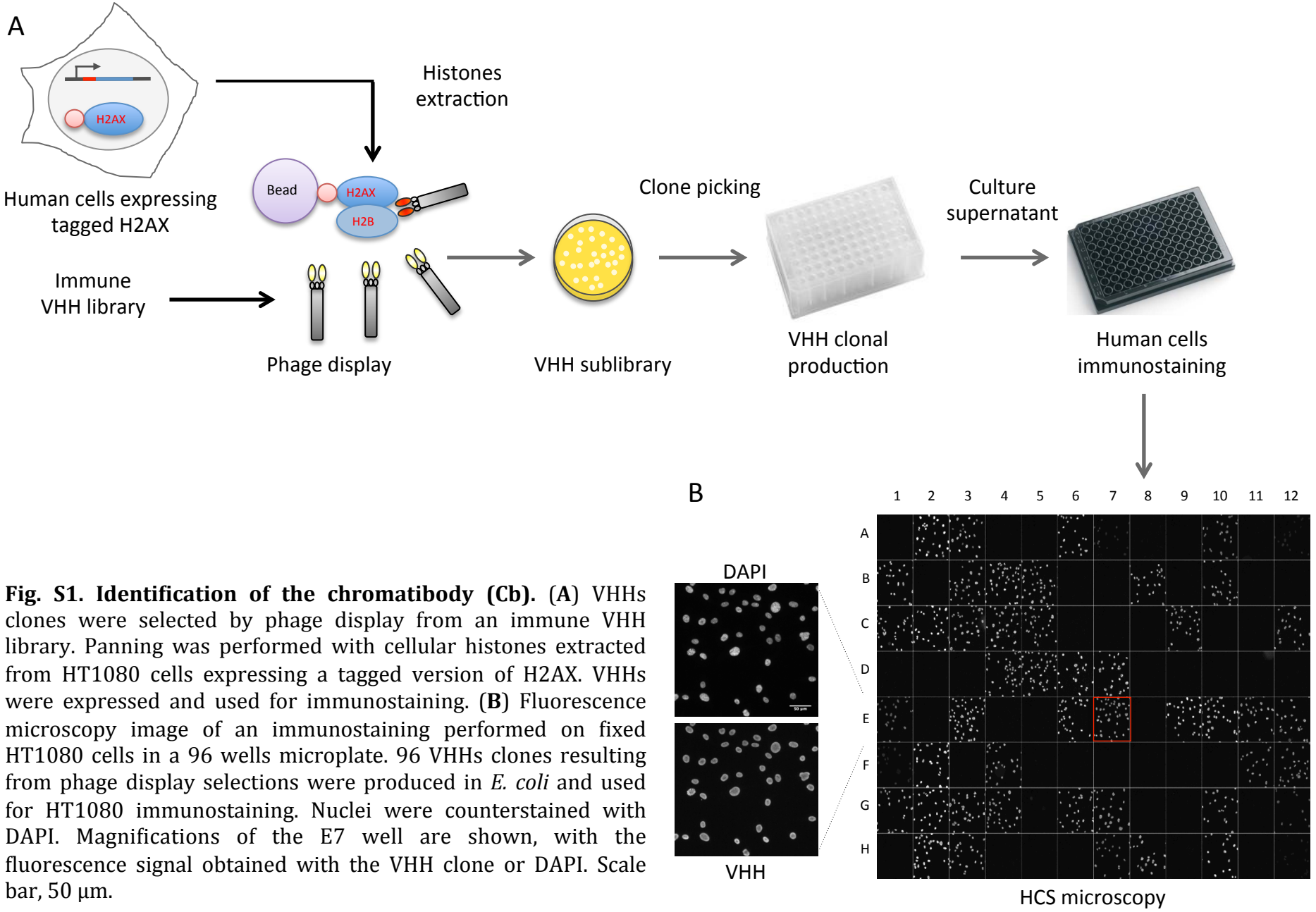


Fig. S1. Identification of the chromatibody (Cb). (**A**) VHHs clones were selected by phage display from an immune VHH library. Panning was performed with cellular histones extracted from HT1080 cells expressing a tagged version of H2AX. VHHs were expressed and used for immunostaining. (**B**) Fluorescence microscopy image of an immunostaining performed on fixed HT1080 cells in a 96 wells microplate. 96 VHHs clones resulting from phage display selections were produced in *E. coli* and used for HT1080 immunostaining. Nuclei were counterstained with DAPI. Magnifications of the E7 well are shown, with the fluorescence signal obtained with the VHH clone or DAPI. Scale bar, 50 µm.

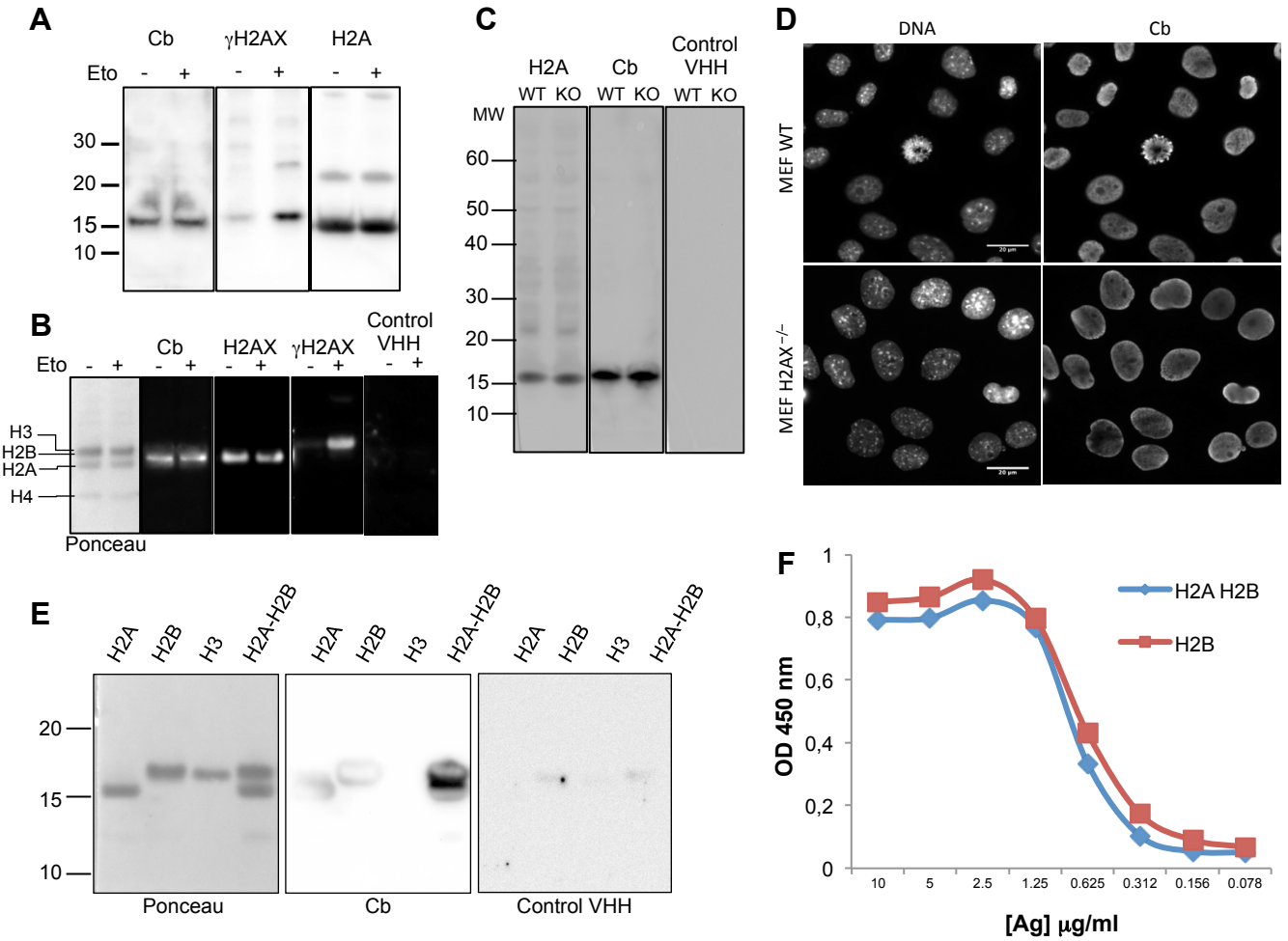


Fig. S2. Characterization of the Cb specificity. (**A**) Whole cell extracts of HCT116 cells, treated or not with Etoposide (Eto), were probed with Cb or anti-γH2AX and anti-H2A antibodies as controls. (**B**) Histones extracted from cells treated or not with etoposide (Eto) were analyzed by western-blot. A ponceau staining of the proteins present on the blot used in the experiment is shown in the left panel (Ponceau). Signals obtained after western-blot with Cb, anti-H2AX, anti-γH2AX or control VHH are presented. (**C**) Whole cell extracts prepared from wild type (WT) or H2AX$^{-/-}$ (KO) MEFs were probed with anti-H2A, Cb or a control VHH. The panels show the signal pattern obtained. (**D**) DNA staining (DAPI) and immunostaining of wild type (WT) or H2AX$^{-/-}$ (KO) MEFs performed with Cb. (**E**) Histones (H2A, H2B or H3) or H2A-H2B dimer were blotted and probed with Cb or a control VHH. A ponceau staining of the blot used in the experiment is shown in the left panel (Ponceau). (**F**) H2B or H2A-H2B dimer were used in an ELISA assay and incubated with anti-H2B antibodies, as positive controls.

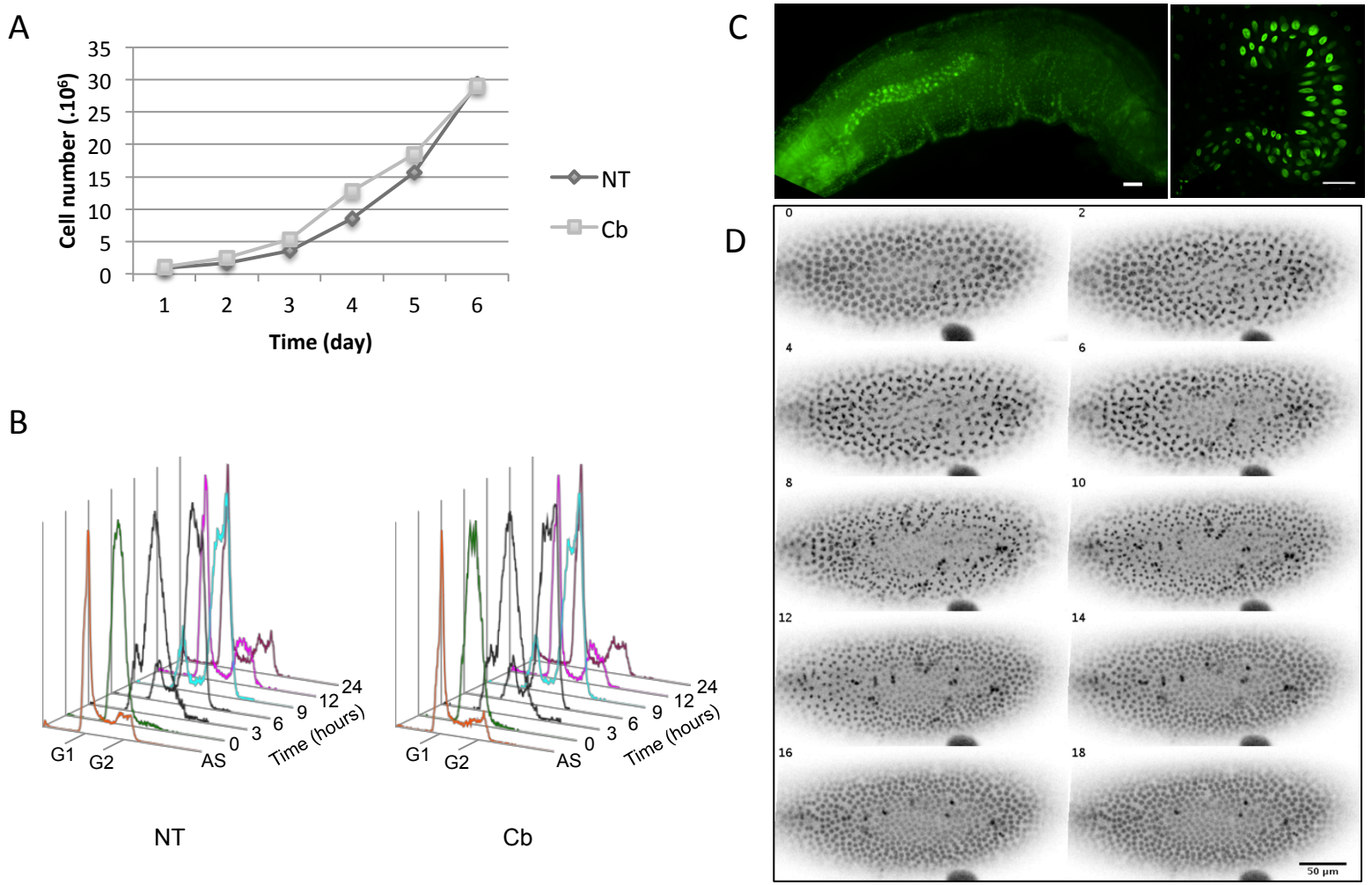


Fig. S3. Cb-mediated chromatin labeling in living systems. (**A**) Growth curves established from parental HT1080 cells (Not Transfected, NT) or HT1080 stably expressing the Cb-GFP (Cb). (**B**) Cell cycle profiles of parental HCT116 cells (NT) or HCT116 cells stably expressing the Cb-GFP (Cb). Cells were synchronized by double thymidine block at G1/S and released in fresh media for the indicated times. AS: asynchronous cells. (**C**) The left panel shows the fluorescence microscopy image of a drosophila living larvae (third instar larvae), with inner structures (in particular the nuclei of the salivary gland). The tubulin promoter was used. Scale bar, 50 µm. The right panel shows the magnification of the dissected salivary gland. Scale bar, 50 µm. (**D**) Time-lapse images of drosophila embryonic development. The Cb is expressed under the tubulin promoter. Time lapse confocal images of a live blastoderm embryo progressing through the 13th cell division of embryogenesis. Time series was acquired with an interval of 2 minutes as indicated. Scale bar, 50 µm.

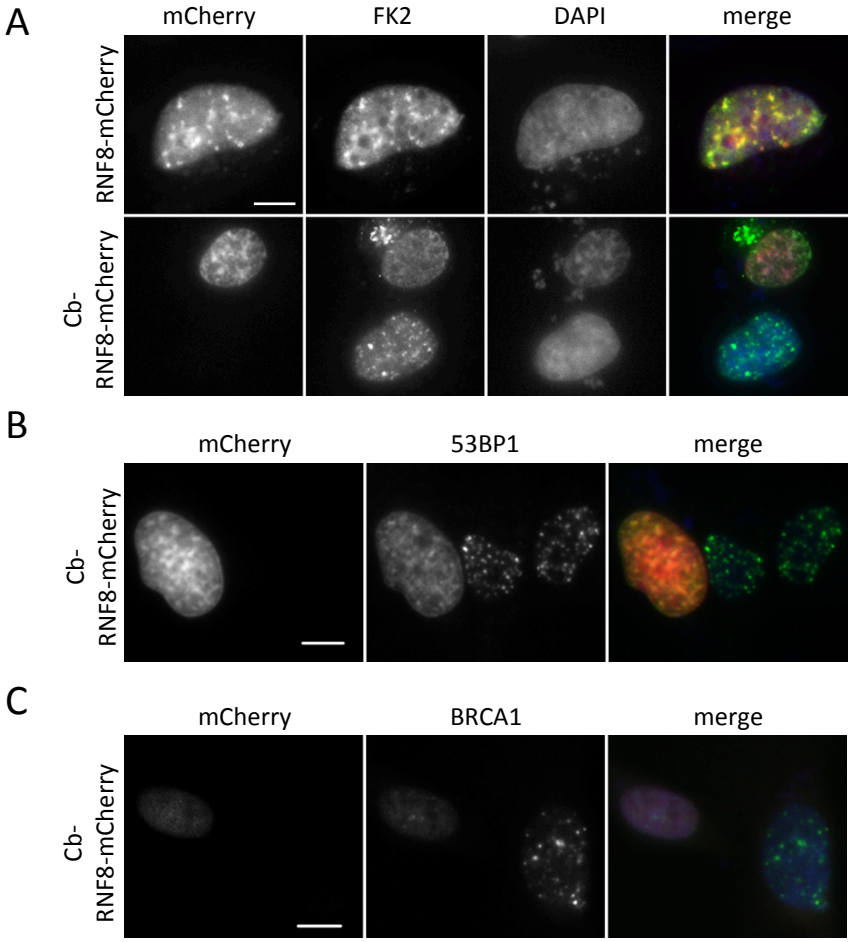
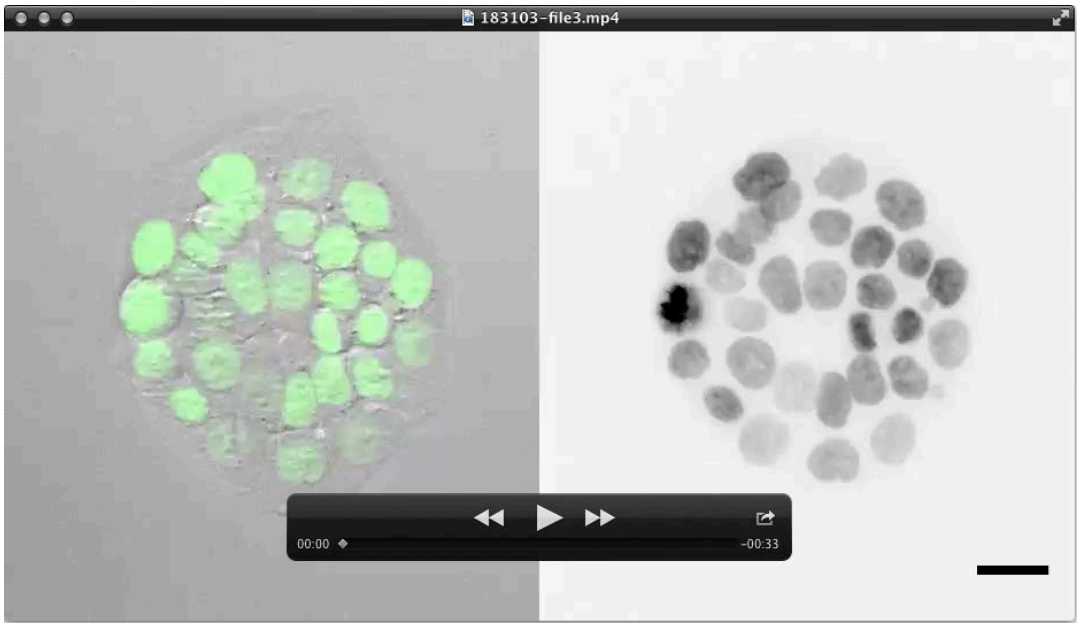


Fig. S4. Cb-driven DDR alteration. (**A**) FK2 ionizing radiation-induced nuclear foci (IRIF) formation in HeLa cells. HeLa cells were transfected with the indicated forms of RNF8 for 24h, exposed to 2 Gy and fixed before being immunostained with an antibody directed against ubiquitin conjugates (FK2) and counterstained with DAPI. The left panel (mCherry) shows the transfected cells, while the non-transfected cells are used as internal control. The right panel shows the merge of the mCherry (red), FK2 (green) and DAPI (blue) signals. (**B**) 53BP1 recruitment to DSBs and foci formation. HT1080 cells were transfected with Cb-RNF8-mCherry (left panel) for 24h, treated 1h (10 pM calicheamicin) and fixed before being immunostained with an antibody directed against 53BP1 (green) and counterstained with DAPI (blue). (**C**) BRCA1 foci formation. HT1080 cells were transfected with Cb-RNF8-mCherry (left panel) for 24h and fixed, without genotoxic treatment, before being immunostained for BRCA1 (green) and counterstained with DAPI (blue). Scale bars, 20 µm.

Table S1. Primers, parental plasmids and purpose of the constructs in this study.

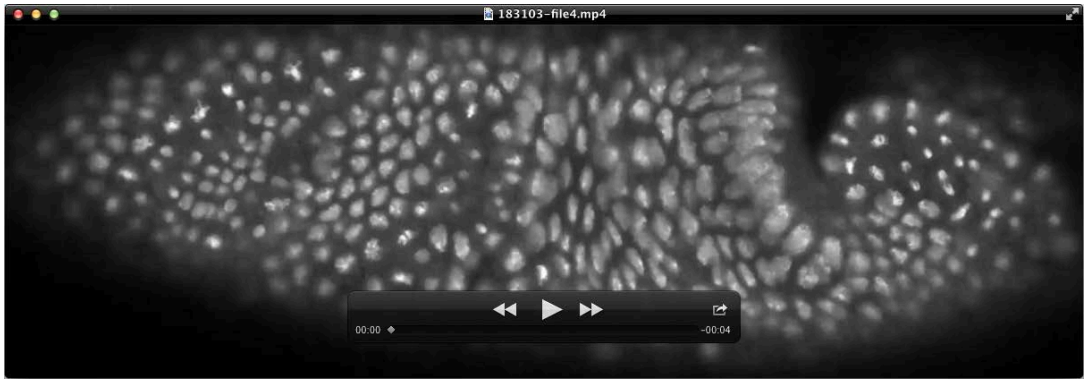
	Primers (5' to 3')	Parental plasmid / Purpose
1	GGCCGCTACCCGTACGACGTTCCGGACTACGCACTCGAGCATCATCATCATCATCATTA G	pHEN4 / HA-His_6 fusion insertion
2	AATTCTAATGATGATGATGATGCTCGAGTGCGTAGTCCGGAACGTCGTACGGGTA GC	pHEN4 / HA-His_6 fusion insertion
3	CAGCCGGCTCGAGCCCAGGTGCAG	pEGFP-N1 / Cb cloning and Cb-Cb-C
4	AATGGATCCTATGCGTAGTCCGGAACGTCGTA	pEGFP-N1 / Cb cloning
5	AGCTTGCCCACCACAGCGAGGACCCCAGCAGCAAGGCTCCCAAGGCGCCCATGGCCCA GGTGCAGTTGCA	pEGFP-N1-Cb / linker insertion
6	ACTGCACCTGGGCCATGGGCGCCTTGGGAGCCTTGCTGCTGGGGTCCTCGCTGTGGTGG GCA	pEGFP-N1-Cb / linker insertion
7	ATTAAGCTTGCTGGAGACGGTGACCAGGGT	pEGFP-N1-Cb / Cb cloning and Cb-C
8	CAGCCGGCTCGAGCCCAGGTGCAG	pEGFP-C1 / Cb cloning
9	AATGGATCCTATGCGTAGTCCGGAACGTCGTA	pEGFP-C1 / Cb cloning



Movie 1. Live imaging of HCT116 cells stably expressing the Cb-GFP fusion.

Time lapse imaging of proliferating HCT116 cells stably expressing the chromatibody-GFP probe (related to Fig. 3A and 3B) showing the performance of chromatibody to visualize, in a non-invasive way, the dynamics of chromatin in living cells. Left and right panel shows fluorescence and transmitted light, respectively. The fluorescent chromatibody probe remains associated to chromatin throughout the cell division cycle and does not interfere with progression through mitosis. Images were acquired sequentially (GFP fluorescence and transmitted light) using an inverted wide field microscope (Zeiss Axio Observer Z1), with a 40x 0.95 NA objective, controlled with the MetaMorph software (Molecular Devices). The acquisition frequency is 5 min.

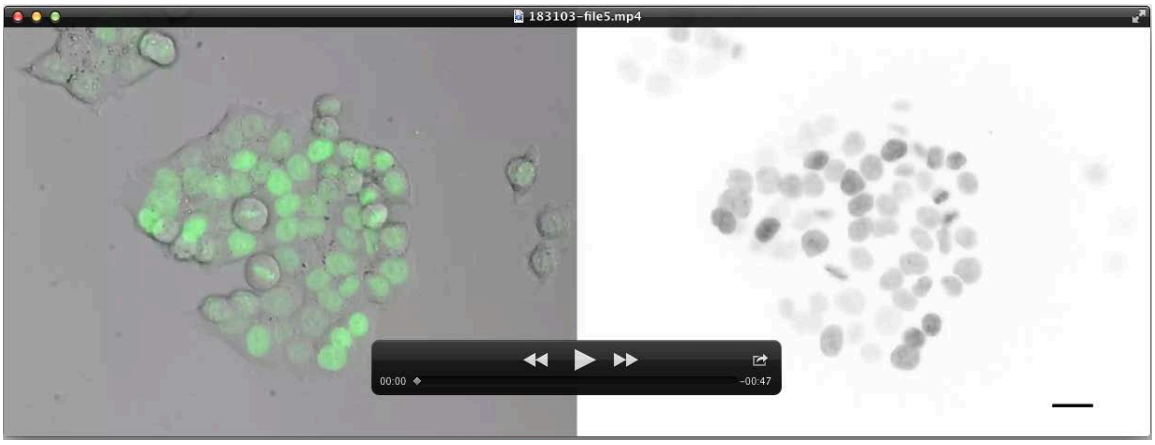
Movie 1 is available at: figshare.com/s/372e901295df11e5a34206ec4bbcf141



Movie 2. Developing drosophila embryo expressing the Cb-GFP fusion.

Time lapse imaging of a drosophila embryo ubiquitously expressing the Cb-GFP probe based on Fig. 3C (left panel). The movie demonstrates the performance of Cb-based fluorescent probe to image in a non-invasive fashion chromatin in living drosophila. GFP fluorescence was acquired using a laser scanning confocal microscope (Zeiss LSM510), with a 25x 0.8 NA objective, driven with the Zen software (Zeiss). Frames were taken every 2 minutes at a constant temperature of 25°C.

Movie 2 is available at: figshare.com/s/5f95973a95df11e5ae3e06ec4bbcf141



Movie 3. HCT116 cells stably expressing the bivalent Cb.

Time lapse imaging of proliferating HCT116 cells stably expressing the bivalent Cb-Cb-GFP probe (related to Fig. 4) showing how a bivalent Cb fluorescent probe perform in the staining of chromatin in living cells. Live cell imaging was performed as described in movie 1.

Movie 3 is available at: figshare.com/s/aff3227495df11e5bc2f06ec4bbcf141